(54) Title: MICROSCOPY IMAGING APPARATUS AND METHOD

(57) Abstract

An object is illuminated by a light source (10) and a periodic pattern of transparent and non–transparent stripes is superimposed onto the object (O). At least three images are recorded at different spatial phases of the pattern by means of a microscope of shallow focal depth, and a three–dimensional image containing only in–focus detail is then derived from the recorded images by image processing which removes the periodic pattern. An illumination mask (14) or the interference fringes of two coherent beams generate the periodic pattern. The different spatial phases are generated by shifting the mask or adjusting the temporal phase difference of the coherent beams.

MICROSCOPY IMAGING APPARATUS AND METHOD

The present invention relates to microscopy imaging apparatus and an imaging method, for generating an image which generally contains only in-focus detail and a method thereof. The present invention is suited to generating images which may be used to create in-focus three-dimensional images of volume structures.

With a conventional light microscope three-dimensional structures cannot be successfully imaged. The resultant image consists of a sharp image of an in-focus region of a three-dimensional structure as well as defocused images of the structures above and below the in-focus region. A conventional light microscope is unable to reject out-of-focus detail.

Confocal microscopes have been developed which optically section a three-dimensional structure to provide in-focus images of individual layers or strata of the structure which can be subsequently combined to form an in-focus three-dimensional image of the complete volume structure. Unfortunately, the light budget of confocal microscopes is generally poor when incoherent light sources are used. Laser scanning confocal microscopes can achieve a very shallow depth of focus, but require expensive apparatus and an illuminating/imaging pinhole through which the laser light must be focused.

In US 5381236 an optical sensor is described which is used to determine the range (distance) of individual features of a three-dimensional structure. The sensor has a periodic patterned light source which illuminates the structure and is reversible (i.e. the pattern is phase shifted 180°). An array of detector elements which are aligned to the pattern of the light source is used to detect an image of the pattern and the reversal of the pattern illuminating the structure. As the pattern will only be imaged well on those parts of the structure which are themselves in focus, this enables the range (distance) of in-focus parts of the structure to be determined. The apparatus and method described in US 5381236 has the disadvantage that in order to work the individual elements of the detector must be exactly aligned with and matched to the pattern of the light source. In practice this has been found to be almost impossible to achieve.

The present invention seeks to provide microscopy imaging apparatus and an imaging method to produce images which, in a similar way to confocal images, comprise substantially only in-focus detail and which can be used to create a three-dimensional image of a structure through optical sectioning of the structure. The present invention achieves the optical sectioning without the need for precise alignment or matching of detector and pattern components and provides at the same time a favourable light budget.

In one aspect, the present invention provides a method of generating an image of a specimen comprising the steps of illuminating the specimen with a light source; generating a substantially periodic spatial pattern on the specimen; recording a first image of the specimen; altering the spatial phase of the pattern on the specimen and recording a second image of the specimen; repeating at least once more the step of altering the spatial phase of the pattern on the specimen and recording a third image of the specimen, the spatial phase of the pattern in at least three recorded images of the specimen being different; and analysing the three or more recorded images of the specimen to remove the spatial pattern from the images thereby to generate an optically sectioned image of the specimen.

While the identified prior art relies on a matched detector grid aligned to stringent requirements with the mask pattern, the present invention is of advantage in that the need for such a matched detector grid is eliminated. Processing of image data is simple and the invention enables production of optically sectioned images from a conventional microscope in real time.

In a preferred embodiment the above method is repeated at different focal positions to produce an in-focus three-dimensional image of the specimen. In a specific embodiment of the invention, described in detail below, a three-dimensional image of the surface texture of a specimen is thereby obtained.

The spatial phase of the pattern may be altered continuously or in discrete steps. Where the spatial phase is altered continuously, the recorded images of the specimen are integrated over a predetermined time period. It has been calculated and also confirmed in practice that high

- 3 -

quality images are obtained when the spatial phase of the pattern is continuously altered. Thus, a wide range of means for altering the spatial phase are of application to the present invention.

In a further aspect, the present invention provides a method of processing image data of at least three images of the same specimen, the images having a substantially periodic pattern superimposed thereon, the spatial phases of the pattern on three images being different, comprising analysing the data so as to generate a composite image, free of the pattern.

In a still further aspect, the present invention provides microscopy imaging apparatus comprising a light source; patterning means for generating a substantially periodic spatial pattern; focusing means for focusing light from the light source on a specimen and generating the pattern on the specimen; phase shift means for adjusting the spatial phase of the pattern generated on the specimen; a detector for detecting images of the specimen; and an analyzer having means for analysing images of the specimen, the spatial phase shift of the pattern being different in at least three images, and means for removing the spatial pattern from the three images of the specimen thereby to generate an optically sectioned image of the specimen.

Preferably, the patterning means is in the form of a mask having one-dimensional local periodicity and the mask pattern is projected onto the specimen. For example, the mask may be a linear grating. The mask may also be a circular mask containing a spiral grating. In this latter case the pattern is conveniently projected onto the specimen by illuminating the specimen through a portion of the grating located towards or at the edge of the circular mask; for in this portion, the spiral grating approximates to a grating of parallel lines. An advantage of this spiral grating is that continuous movement of the grating is achieved by rotation of the mask. Alternatively, where a coherent light source is employed, the patterning means may be provided by a second coherent light source so arranged to interfere with the light from the first light source.

In a further aspect, the present invention provides apparatus adapted to modify a conventional

microscope, comprising patterning means for generating a substantially periodic spatial pattern, phase shift means for adjusting the spatial phase of the pattern and an analyzer having means for analysing images of a specimen on which the spatial pattern has been generated, the spatial phase shift of the pattern being different in at least three images, and means for removing the spatial pattern from the three images thereby to generate an optically sectioned image of the specimen.

In a further embodiment, the present invention provides a method of adapting a conventional microscope to produce optically sectioned images of a specimen, the method comprises introducing patterning means into the optical system of the microscope for generating a substantially periodic spatial pattern on the specimen; providing spatial phase shift means for adjusting the spatial phase of the pattern to produce at least three different spatially phase shifted patterns on the specimen; and providing an analyzer having means for analysing at least three separate images of the specimen, each with a different spatial phase shift of the pattern, and means for removing the spatial pattern from the images of the specimen thereby to generate optically sectioned image of the specimen.

It is thus an advantage of the present invention that a conventional microscope, a ubiquitous piece of laboratory equipment, may readily be converted so as to be capable of providing optically sectioned images.

The present invention also provides a method of processing image data of at least three images of the same specimen, the images having a substantially periodic pattern superimposed thereon, the spatial phases of the pattern on three images being different, comprising analysing the data so as to generate a composite, from said image, free of the pattern.

Embodiments of the present invention will now be described by way of example with reference to the accompanying drawings in which:-

Fig. 1   is a schematic diagram of microscopy imaging apparatus in accordance with the present invention;

Fig. 2   is a schematic diagram of an optical system of the present invention;

Fig. 3   shows measured axial responses of the system of Fig. 2;

Fig. 4(a)   shows an autofocus image of a lily pollen grain (field size is 100μm x 70μm)
                obtained according to the present invention; and

Fig. 4(b)   shows a conventional image of a lily pollen grain (not according to the present
                invention) when the microscope is focused in a mid-depth plane.

The imaging apparatus shown in Fig. 1 has many of the features of a conventional microscope
and in particular includes an incoherent light source 10, focusing means preferably in the form
of one or more lens 11 which focuses light from the light source 10 onto the object to be
imaged O. A beam-splitter 12 is located between the light source and the object so the light
reflected from the object is reflected towards a light detector 13 preferably in the form of a
CCD camera. In addition, the apparatus includes a mask 14 which is patterned, for example
in the form of a grating, and which is provided with the light source 10 so that the object is
illuminated with a non-zero, spatially substantially periodic pattern. The mask pattern is thus
projected onto the specimen. Preferably, the mask pattern has local periodicity in only one
dimension. The detector plane is conjugate to the plane in which the projected pattern is
focused to enable an optically sectioned image of the object to be formed.

The apparatus further includes spatial phase shift means 15 for adjusting the spatial phase of
the mask pattern focused on the object such that at least three different phases of the mask
pattern are generated. The phase shift means 15 may be in the form of a carriage for moving
the mask either incrementally or continuously relative to the object so that the spatial phase
of the patterned light focused on the object can be altered. In the case of a mask which is
in the form of linear grating, the carriage is arranged to move the grating perpendicular to the
axis of the imaging apparatus. Another option for adjusting the spatial phase includes
movement of the beam splitter so as to shift the phase of the projected pattern.

- 6 -

The pattern can be generated on the specimen in different ways. The pattern is optionally generated by employing a ribbon belt carrying a pattern, for example parallel stripes. Here, a light source projects the pattern of the ribbon onto the specimen. The ribbon forms a loop around the light source so that the light only shines through that part of the ribbon that is situated between the light source and the object. Alternatively, the ribbon forms a loop around the specimen.

Another option is that the ribbon forms a loop around a mirror, one part of the ribbon being situated between the light source and the mirror. The light that has passed through the ribbon would then be reflected onto the specimen by means of the mirror. Such a ribbon can thus be moved continuously or incrementally in the same direction at all times. It offers the advantage of generating a pattern of, for example, exactly parallel stripes (as does a mask comprising a rectangular panel) without the necessity of moving it in a sawtooth fashion or reversing the direction of movement.

In Fig. 1 the phase shift means 15 is in the form of a carriage which is arranged to move the mask 14 to three predetermined positions located in a plane perpendicular to the axis of the imaging apparatus (into and out of the page). The three positions for the carriage are chosen so that three different spatial phases of the mask are focused on the object. For example, the three phases may be: ϕ, $\phi+120°$ and $\phi+240°$. Alternative spatial phase shifts may be generated, for example: ϕ, $\phi+90°$, $\phi+180°$ and $\phi+270°$. With each of the two examples given above the angular difference between the individual spatial phases is the same, however is not essential for the angular difference to be the same, i.e spatial phase shifts of ϕ, $\phi+90°$ and $\phi+270°$ may also be used.

The imaging apparatus further includes an analyzer 16 connected to the detector which analyses the output of the detector. The analyzer 16 may include memory means 17, for example in the form of plurality of buffers, which separately store the three or more images of the object each illuminated with different phases of the mask. The analyzer 16 further includes pattern removal means 18 for analysing the three or more stored images so as to remove the mask pattern from the images thereby revealing an optically sectioned image of

the object. 3-D imaging means 19 is conveniently provided which employs standard rendering techniques to generate a three-dimensional image of the object through combining a plurality of different optically sectioned images, generated by the pattern removal means 18, at different focal positions.

The pattern removal means 18 may operate to determine directly the in-focus image minus the fringing. For example, where three images are I_1, I_2, I_3, generated at three equally spaced spatial phases the in-focus image, I, can be determined by using the following equation:

$$I = \sqrt{(I_1 - I_2)^2 + (I_1 - I_3)^2 + (I_2 - I_3)^2}$$

Implementing this procedure for each pixel of the image is, however, computationally intensive. Alternatively, to enable real-time imaging to be performed without the need for dedicated image processing apparatus, the analyzer 16 may include a look-up table connected to the pattern removal means 18. The look-up table is a tabulation of all possible solutions to the above equation with respect to the three input image values and enables the image I to be determined by reference to the table rather than through calculation of the solution of the equation for each pixel.

The imaging apparatus described above takes advantage of the fact that any non-zero spatial frequency in the image of the object attenuates as the apparatus is defocused. This means that the mask pattern is only imaged well on those parts of the object which are in focus and thus provides a means of sectioning those parts of the object, which are in focus, albeit with an undesirable fringe pattern superimposed. By recording three or more images of the object at the same focal position but with different superimposed spatial phases of the pattern it is possible for the images to be analyzed to remove the superimposed patterns to reveal an optically sectioned image of the object.

Preferably, the mask 14 is in the form of a linear grating that is a square wave pattern, since

- 8 -

this gives greatest light throughput. Alternating transparent and non-transparent bands of equal width are suitable. A pattern of wider bands results in a deeper optical section. Good results have been obtained using bands of width 5-30 microns, 12½ microns in particular, though different widths will be appropriate according to the optical sectioning required. However, any applied substantially periodic light intensity pattern may be employed. Suitable alternative masks include a spiral grating. Moreover, two dimensional periodic patterns may also be used but naturally the analysis of the resultant images is somewhat complicated.

As the pattern spacing determines the depth of the optical sectioning, ideally the pattern spacing (spatial periodicity) of the mask is selected and the illumination magnification is arranged so that only the fundamental of the pattern is projected onto the object. In the case of the linear square wave grating described above where the three phases of the grating ϕ, $\phi+120°$ and $\phi+240°$ are projected onto the object, the third harmonic is automatically cancelled out in the subsequent processing of the three images and so it is not essential to avoid the third harmonic being projected on to the object. This provides greater freedom in the overall design of the imaging system and improves the light budget of the apparatus. In general, where the difference between the n phase shifts of the pattern projected onto the object is the same in each case the nth harmonic and harmonics thereof are automatically cancelled out.

Imaging apparatus has been described above in which three or more discrete predetermined phase shifts of the pattern are projected onto the object. Where the spatial phase of the pattern is varied continuously, for example using a rotating spiral mask or continuous movement of a grid-type mask, the images of the object are integrated over predetermined time periods to generate the optically sectioned image.

The imaging apparatus may alternatively employ a coherent light source. This in turn permits the mask to have intensity or phase patterning. The use of period phase patterning is preferred as the light budget of the apparatus is greater than in the case of periodic intensity patterning. In a further alternative, two coherent light beams may be employed which are arranged so that the light from the two sources interfere to generate a fringe pattern in the

object volume.   Where two coherent light beams are used the temporal phase difference between the two beams may be altered to effect the required spatial phase shifts of the fringe pattern. Where one or more coherent light sources are used, the fringe pattern extends in depth through the object volume hence exact axial alignment of the detection plane is no longer critical.   This approach is particularly appropriate for fluorescence imaging.

The optical system of a specific embodiment of the present invention simply consists of an illumination mask, $S(t_0, w_0)$ which is imaged onto an object of amplitude transmittance or reflectance, $\tau(t_1, w_1)$. The final image is recorded by a CCD camera in the image plane (t,w). The mask is illuminated incoherently which permits us to write the image intensity as

$$I(t,w) = \int\int S(t_0,w_0)\left|\int\int h_1(t_0+t_1,w_0+w_1)\tau(t_1,w_1)h_2(t_1+t,w_1+w)dt_1 dw_1\right|^2 dt_0 dw_0 \qquad (1)$$

where $h_{1,2}$ represents the amplitude point spread functions of the two lenses. We also elect to work in optical co-ordinates (t,w) which are related to real co-ordinates (x,y) via $(t,w) = 2\dfrac{\pi}{\lambda}(x,y)n\sin\alpha$ where $n \sin \alpha$ is the numerical aperture (NA) and λ denotes the wavelength.

We now assume that the illumination mask takes the form of a one-dimensional grid which we may write for simplicity as

$$S(t_0,w_0) = 1 + m\cos(\bar{v}t_0 + \phi_0) \qquad (2)$$

where m denotes a modulation depth and ϕ_0 an arbitrary spatial phase. The normalised spatial frequency $\bar{v}$ is related to the actually spatial frequency v via $\bar{v} = \beta\lambda v/NA$ where β denotes

- 10 -

the magnification between the grid plane and the specimen plane. If we now substitute equation (2) into equation (1) we have

$$I(t,w)=I_0+I_C\cos\phi_0+I_S\sin\phi_0 \qquad\qquad (3)$$

where I_0 is given by equation (1) with S=1 and, of course, represents a conventional wide field image. I_c and I_s represent the image due to masks of the form $m\cos(\bar{v}t_0)$ and

$m\sin(\bar{v}t_0)$ respectively. If we now form $I_p=\sqrt{I_C^2+I_S^2}$ we remove the grid pattern from the

image of the specimen. This is achieved by taking three images, I_1, I_2 and I_3 corresponding to the relative spatial phases $\phi_0 = 0$, $\phi_0 = 2\pi/3$ and $\phi_0 = 4\pi/3$ respectively. We thus obtain an optically sectioned image, which does not contain I_0, by calculating

$$I_P=\sqrt{(I_1-I_2)^2+(I_1-I_3)^2+(I_2-I_3)^2}$$

which is analogous to square law detection in communications systems.

In order to demonstrate the ability of our system to produce optically sectioned images in real time we introduced a 40 lines/mm one dimensional grid into the illumination path of a conventional microscope. Since we used infinity tube lengths optics it was necessary to introduce separate lenses both to project the image of the grid onto the specimen and to image the specimen onto the CCD camera. This resulted in an effective magnification between the illumination plane and the specimen of (50/180)M where M is the nominal magnification of the objective lens. A 15W tungsten-halogen lamp was used at the light source together with a green filter (bandwidth 100nm). Images were recorded with a CCD camera and transferred to a Matrox Meteor frame grabber. The grid was moved in a simple saw tooth fashion synchronised to the camera frame rate such that any three successive camera images

correspond to a spatial shift of one third of a period in the position of the projected image of the grid, Figure 2. The fact that the grid is in motion during the integration time of each frame results in a reduction in the valves of I_c and I_s in equation (3) by a factor of sinc ($\pi t/3T$) where t is the integration of the camera and T the time between the recording of successive frames. In our case $T=2t=40$ms and hence this factor is only 0.955. In the worst case, $T=t$, the factor falls to only 0.827. The optically sectioned images were obtained using equation (4) together with a look up table which mapped all possible combinations of I_1, I_2 and I_3 from our 8 bit camera to I_p.

In order to measure the optical sectioning strength of our microscope we used a plane mirror together with a calibrated axial translation stage; the resulting axial responses are shown in Figure 3 for two Olympus MD Plan objectives. We note that the use of the 50X 0.75 NA corresponds to $v = 0.4$ in our system for which we predict a full width half maximum (FWHM) of 0.87μm which compares well with the measured value of 0.91μm. Theory predicts that the 150X, 0.95NA dry objective should give a $v = 0.8$ and hence a FWHM of 0.27μm. In practice we measure 0.43μm which corresponds to a numerical aperture of this lens is 0.85 rather than 0.95. This discrepancy is consistent with other measurements performed with high aperture lenses and may be due to a variety of causes one of which is likely to be the strong attenuation of the marginal rays which leads to an effective apodisation of the pupil function.

In Figure 4 we show images by imaging the thick volume structure of a lily pollen grain. Figure 4(a) represents an autofocus image obtained by displaying the maximum image intensity at each pixel throughout a 30μm axial scan according to the present invention using the 50X, 0.75 NA objective lens. The full surface texture of the grain is well resolved over the entire image volume. Figure 4(b), on the other hand shows a conventional image taken at a plane midway through the grain. It is clear that a good deal of out-of-focus blur is present here which prevents any meaningful three-dimensional image processing being carried out.

It will be apparent that a conventional microscope may be easily modified so as to generate

optically sectioned images. The modification consists of introducing the patterned mask and carriage into the illumination system of the microscope and the addition of the analyzer, described above. The introduction of the mask and carriage may in turn require the introduction of additional optics, however, where the conventional microscope includes an iris focused on the object, the iris may simply be replaced by the mask and carriage without further alteration.

The imaging apparatus described above may be employed in a wide range of imaging modalities including, for example, darkfield and differential interference contrast. Also, the imaging apparatus may be used in all conventional microscopy applications. In particular the apparatus may be used in biomedical applications and has the advantage that optically sectioned fluorescent imaging may be performed efficiently without the need for a laser as the light source.

CLAIMS

1. A method of generating an image of a specimen comprising the steps of illuminating the specimen with a light source; generating a substantially periodic spatial pattern on the specimen; recording a first image of the specimen; altering the spatial phase of the pattern on the specimen and recording a second image of the specimen; repeating at least once more the steps of altering the spatial phase of the pattern on the specimen and recording a third image of the specimen, the spatial phase of the pattern in at least three recorded images of the specimen being different; and analysing the three or more recorded images of the specimen to remove the spatial pattern from the images thereby to generate an optically sectioned image of the specimen.

2. A method according to Claim 1 wherein two coherent light sources are used for illuminating the specimen and for generating a substantially periodic spatial pattern on the specimen.

3. A method according to Claim 1 comprising using a mask to project the pattern onto the specimen.

4. A method according to Claim 3 wherein said mask has local periodicity in only one direction.

5. A method according to Claim 3 or Claim 4 wherein said mask comprises substantially parallel stripes of varying opacity.

6. A method according to Claim 4 wherein said mask comprises stripes extending in a radial direction.

7. A method according to Claim 5 or Claim 6 wherein in said mask substantially transparent stripes alternate with substantially non-transparent stripes.

- 14 -

8.   A method according to Claim 5 or Claim 6 wherein the opacity of said stripes varies smoothly in a direction in which a periodicity can be found.

9.   A method according to Claim 3 or Claim 4 wherein said mask has a spiral pattern.

10.   A method according to any of Claims 3 to 9 wherein said step of altering the spatial phase of the pattern comprises moving said mask.

11.   A method according to any of Claims 3 to 8 wherein said step of altering the spatial phase of the pattern comprises moving said mask in a direction in which a periodicity can be found.

12.   A method according to any of Claims 1 to 11 wherein the spatial phases of three recorded images are $\varphi_{\circ}$, $\varphi_{\circ}$ +120° and $\varphi_{\circ}$ +240° respectively, $\varphi_{\circ}$ being an arbitrary phase.

13.   A method according to any of Claims 1 to 12 comprising substantially continuously altering the spatial phase of the pattern.

14.   A method according to any of Claims 1 to 12 comprising substantially incrementally altering the spatial phase of the pattern.

15.   A method of processing image data of at least three images of the same specimen, the images having a substantially periodic pattern superimposed thereon, the spatial phases of the pattern on three images being different, comprising analysing the data so as to generate a composite image, free of the pattern.

16.   A method according to Claim 15 wherein the pattern has local periodicity in only one direction.

17.   Microscopy imaging apparatus comprising a light source; patterning means for

- 15 -

generating a substantially periodic spatial pattern; focusing means for focusing light from the light source on a specimen and generating the pattern on the specimen; phase shift means for adjusting the spatial phase of the pattern generated on the specimen; a detector for detecting images of the specimen; and an analyzer having means for analysing at least three images of the specimen, the spatial phase shift of the pattern being different in the at least three images, and means for removing the spatial pattern from the three images of the specimen thereby to generate an optically sectioned image of the specimen.

18.   Apparatus according to Claim 17, wherein said patterning means comprises two coherent light sources for illuminating the specimen and for generating a substantially periodic pattern on the specimen.

19.   Apparatus according to Claim 17 wherein said patterning means comprises a mask, and means for projecting the pattern onto the specimen.

20.   Apparatus according to Claim 19 wherein said mask has local periodicity in only one direction.

21.   Apparatus according to Claim 19 or Claim 20 wherein said mask comprises substantially parallel stripes of varying opacity.

22.   Apparatus according to Claim 20 wherein said mask comprises stripes extending in a radial direction.

23.   Apparatus according to Claim 21 or Claim 22 wherein in said mask substantially transparent stripes alternate with substantially non-transparent stripes.

24.   Apparatus according to Claim 21 or Claim 22 wherein the opacity of said stripes varies smoothly in a direction in which a periodicity can be found.

- 16 -

25.   Apparatus according to any of Claims 21 to 24 wherein said stripes are between 5 and 30 microns wide.

26.   Apparatus according to Claim 19 or Claim 20 wherein said mask has a spiral pattern.

27.   Apparatus according to any of Claims 19 to 26 wherein said phase shift means is adapted to move said mask.

28.   Apparatus according to any of Claims 19 to 25 wherein said phase shift means is adapted to move said mask in a direction in which periodicity can be found.

29.   Apparatus according to any of Claims 17 to 28 wherein said phase shift means is adapted to shift the phase of the pattern in a substantially continuous way.

30.   Apparatus according to any of Claims 17 to 28 wherein said phase shift means is adapted to shift the phase of the pattern in a substantially incremental way.

31.   Apparatus according to any of Claims 17-30 comprising a detector for detecting fluorescence images of said specimen.

32.   Apparatus adapted to modify a conventional microscope into apparatus according to any of Claims 17-31, comprising patterning means for generating a substantially periodic spatial pattern, phase shift means for adjusting the spatial phase of the pattern and an analyzer having means for analysing images of a specimen on which the spatial pattern has been generated, the spatial phase shift of the pattern being different in at least three images, and means for removing the spatial pattern from the three images thereby to generate an optically sectioned image of the specimen.

33.   A method of adapting a conventional microscope to produce optically sectioned images of a specimen, comprising the steps of introducing patterning means into the optical system of the microscope for generating a substantially periodic spatial pattern

on the specimen; providing spatial phase shift means for adjusting the spatial phase of the pattern to produce at least three different spatially phase shifted patterns on the specimen; and providing an analyzer having means for analysing at least three separate images of the specimen, each with a different spatial phase shift of the pattern, and means for removing the spatial pattern from the images of the specimen thereby to generate optically sectioned image of the specimen.
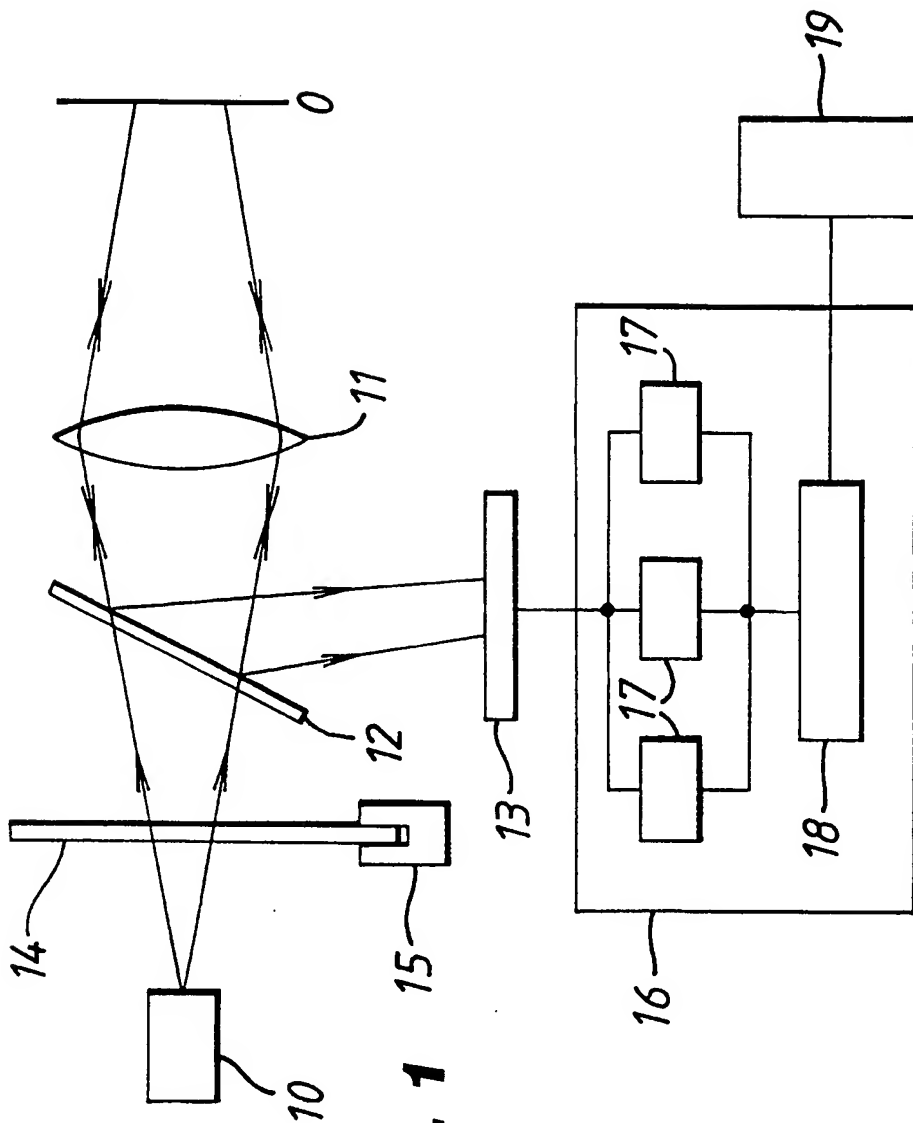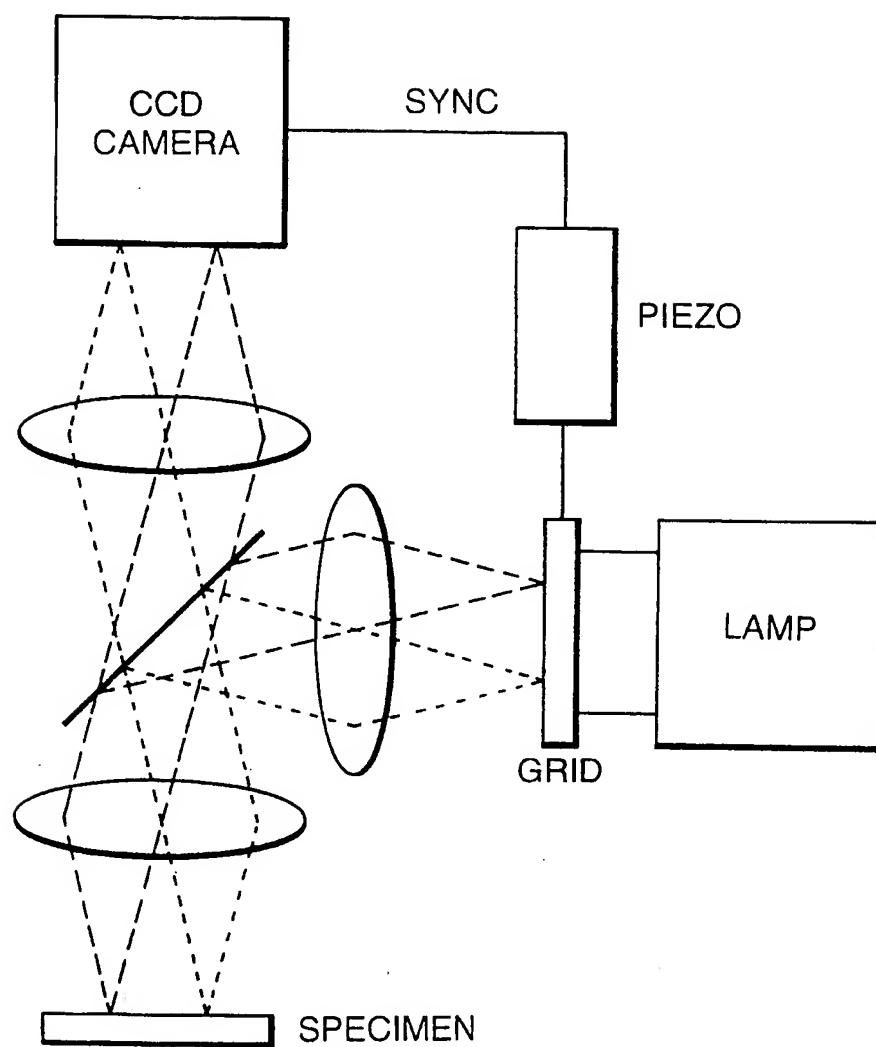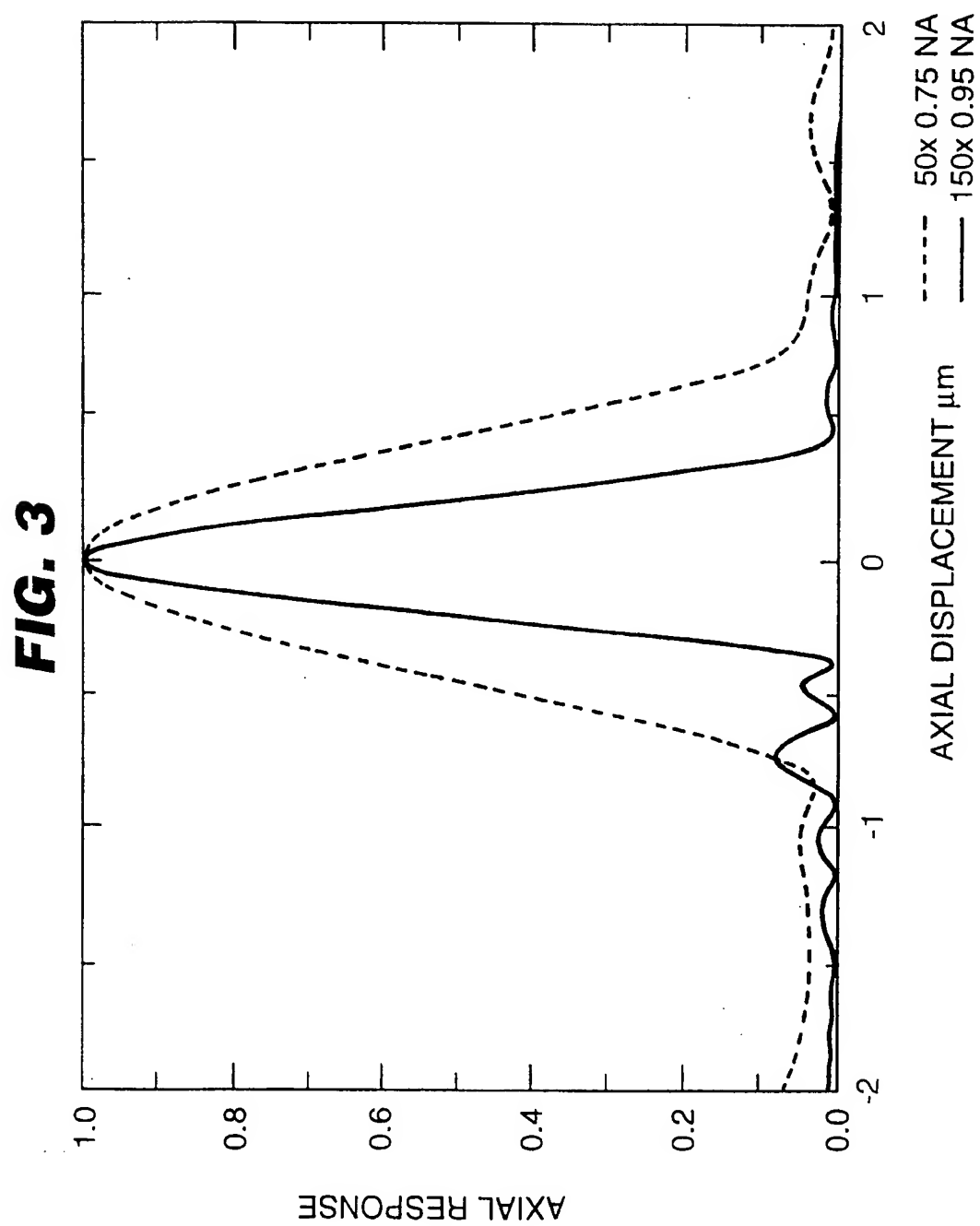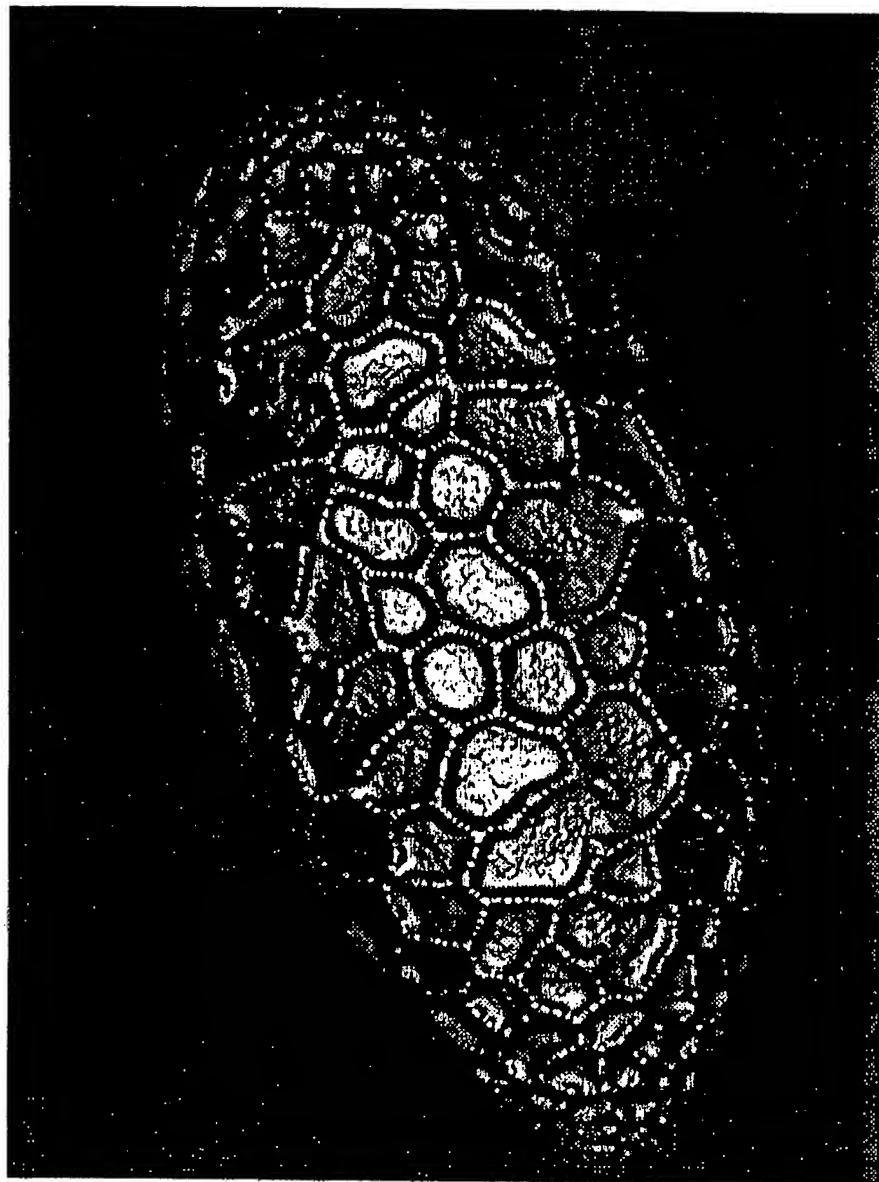
FIG. 1

FIG. 2

FIG. 3

FIG. 4(a)

FIG. 4(b)

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 G02B21/00 G06T5/50 G01B11/24

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G02B G06T G01B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document. with indication. where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 06509 A (GIM SYSTEMS LTD ;FRIEDMAN MARK M (IL); BEN LEVY MEIR (IL); PELEC E) 20 February 1997 see page 6, line 18 - page 8, last line see page 9, line 21 - page 11, last line; figure 2 see page 13, line 15 - page 16, last line	1-4,10, 17-20, 27-33
X	GRUBER M ET AL: "SIMPLE, ROBUST AND ACCURATE PHASE-MEASURING TRIANGULATION" OPTIK, vol. 89, no. 3, 1 January 1992, pages 118-122, XP000247177 see page 121, left-hand column, paragraph 3; figures 6,8 see page 122, left-hand column	15

-/--

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 July 1998	07/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Ciarrocca, M

1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate. of the relevant passages	Relevant to claim No.
A	US 5 381 236 A (MORGAN COLIN G) 10 January 1995 cited in the application see column 6, line 36 - column 7, line 12 see column 9, line 6 - line 17 see column 10, line 33 - line 48; figures 8A,B ---	1,3-5, 10,11, 14-17, 19-21, 27,28, 30,31,33
A	US 5 513 275 A (KHALAJ BABAK H ET AL) 30 April 1996 see column 6, line 14 - line 23 see column 7, line 8 - line 9; figures 1,5 -----	15

INTERNATIONAL SEARCH REPORT

Information on patent family members

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9706509	A	20-02-1997	AU	6680296 A	05-03-1997
			EP	0842497 A	20-05-1998
US 5381236	A	10-01-1995	AU	1195892 A	07-09-1992
			DE	69207176 D	08-02-1996
			DE	69207176 T	04-07-1996
			EP	0571431 A	01-12-1993
			WO	9214118 A	20-08-1992
			JP	6505096 T	09-06-1994
			JP	6506287 T	14-07-1994
US 5513275	A	30-04-1996	NONE		